Preclinical development of biology-based therapeutic strategies for aggressive pediatric brain tumors

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SUMMARY

Diffuse intrinsic pontine glioma (DIPG) and other diffuse midline gliomas (DMG) are highly malignant pediatric brain tumors, for which no effective therapy exists. Failure of therapeutic interventions is caused by three factors: the delicate location of the tumor that makes it ineligible for surgical intervention, the intrinsic resistance of the tumors to chemo- and radiotherapy and the presence of an intact blood-brain barrier, preventing therapeutic drugs from reaching the tumor site. Moreover, the lack of tumor material for research and the paucity of high-quality studies into these diseases play a significant role in the unavailability of an effective treatment.

Atypical teratoid/rhabdoid tumors (AT/RT) are another type of aggressive pediatric brain tumors, often diagnosed in the first year of life. For these tumors surgical intervention is often an option, and chemotherapy may have a significant antitumor effect. Nonetheless, these tumors often relapse and eventually lead to death in the vast majority of children. As with many other pediatric brain tumors, very little tumor material is available for research, and only a handful of preclinical therapeutic studies have been published to date.

Chapter one introduces the pediatric brain tumors that form the subject of the research presented in this thesis – DIPG, DMG and AT/RT- and summarizes the basic knowledge on these cancers as it existed at the start of our studies.

The mesenchymal transition is a molecular process involved in therapy resistance in cancer. **Chapter two** provides an overview of the signaling pathways involved in the initiation and maintenance of the mesenchymal transition in pediatric high grade glioma (pHGG, including DIPG). The review summarizes current basic molecular knowledge on these pathways and applies this knowledge to the biology of pHGG, thereby providing a rationale for new avenues of research to intervene in the therapy-resistant phenotype of these tumors.

Cell culture is an essential technique to study any type of cancer and to test novel therapeutics. In **chapter three** we describe the influence of culture methods on the response of DIPG cells to novel targeted therapies. We show significant differences in signaling pathway activation, gene expression and resulting drug susceptibility in DIPG cells, between the different available culture methods. As such, **chapter three** encourages researchers to cautiously interpret *in vitro* results in DIPG studies, and to combine multiple model systems to ensure the translational relevance of their findings.

In our studies we encountered difficulties in applying basic laboratory methods, most importantly transgene expression and RNA interference, to cultured DIPG and pHGG cells. In **chapter four** we describe a method to reliably perform lentiviral transduction on these cells, thereby overcoming some of these difficulties.

In **chapter five** we describe the discovery of the maternal embryonic leucine zipper kinase (MELK) as a therapeutic target in DIPG, and the MELK inhibitor OTSSP167 as a potent antitumor agent in DIPG cells. Combining next generation sequencing and a variety of laboratory techniques we identify the peroxisome proliferator-activated receptor gamma (PPARγ) as a downstream effector of MELK in DIPG, involved in the antitumor effect of OTSSP167 on DIPG cells. We show that OTSSP167 is incapable of crossing the blood-brain barrier (BBB), disqualifying it for use in clinical trials, except perhaps when locally administered or combined with BBB-disrupting interventions. Nonetheless, we show a strong antitumor effect of OTSSP167 in xenograft models of DIPG established in mice

lacking drug transporters on the BBB, encouraging the development of BBB-penetrable MELK inhibitors and the exploration of the possibility to administer OTSSP167 locally.

Based on the efficacy of MELK inhibition in DIPG, we evaluated its antitumor potential in AT/RT in **chapter six**. We show that, like DIPG, AT/RTs overexpress MELK and demonstrate antitumor efficacy of OTSSP167 in AT/RT cells. Combining OTSSP167 in these cells with inhibitors of components of the mitogen-activated protein kinase (MAPK) pathway – known to be activated in AT/RT- results in a synergistic antitumor effect in AT/RT cells. Vascular phenotyping of AT/RT xenografts and tumor samples reveals significant structural and functional BBB deficiencies in these tumors. As a result, combined MEK/MELK inhibition prolonged survival of mice bearing AT/RT xenografts, showing that non-BBB penetrable drugs may also play a role in developing a therapeutic regimen for AT/RT.

As a sequel to **chapter two**, **chapter seven** describes the development of a therapeutic strategy aimed at reversing the mesenchymal phenotype of DIPG cells, thereby rendering them susceptible to other therapeutic modalities, such as radiotherapy. We identify the receptor tyrosine kinase AXL as a driver of the mesenchymal transition in DIPG, and show that combined inhibition of AXL and histone deacetylases (HDACs) synergistically and selectively reduces the viability, invasion and migration of DIPG cells. Next generation sequencing studies, combined with an array of other molecular techniques, shows that combined AXL/HDAC inhibition synergistically reduces the mesenchymal, stem cell-like phenotype of DIPG cells. Moreover, AXL/HDAC inhibition significantly downregulates the expression of DNA repair genes, sensitizing DIPG cells to ionizing radiation. We demonstrate that the AXL inhibitor BGB324 and HDAC inhibitor panobinostat cross the BBB. Consequently, treatment of DIPG xenograft- or allograft-bearing mice with BGB324 and panobinostat – either systemically or via convection-enhanced delivery- significantly prolonged their survival. As such, we propose that combined AXL/HDAC inhibition can form the backbone of a future multimodal therapeutic strategy for DIPG which includes radiotherapy.

Chapter eight summarizes the therapeutic targets identified and proposed in preclinical studies of DMG – including DIPG- over the past years. It reflects the vast increase in our knowledge of the biology of these tumors, while at the same time critically evaluating the resulting therapeutic targets. We conclude that despite all international efforts, none of these discoveries has led to a successful therapeutic intervention yet, underlining the need for more translationally oriented studies on DMG.

Chapter nine provides a general scientific discussion of the work presented in this thesis, whereas **chapter ten** represents my personal vision on the past and future of translational research into aggressive pediatric brain tumors.

In summary, DMG and AT/RT remain among the most difficult-to-treat, lethal pediatric cancers. Despite a massive increase in knowledge on the biology of these tumors, no effective therapy has yet been developed. With the research presented in this thesis we aim to contribute to the knowledge of these tumors from a translational perspective, developing preclinical and clinical therapeutic strategies from a combined biological and clinical rationale. Given the results we obtained in the past years we are optimistic that, although it will be a difficult road, we now have the tools, knowledge, collaborations and will to develop a curative treatment for DMG and AT/RT, and we hope that our own research will play a valuable part in this development.

CLOSING REMARKS

The development of effective and safe therapeutic strategies for pediatric brain tumors represents the greatest challenge in research in pediatric oncology. This is especially true for DIPG, as its critical location, diffuse growth pattern, intrinsic therapy resistance and maintenance of an intact BBB preclude the effective use of the vast majority of our therapeutic arsenal. Nonetheless, the relentless efforts of the international pediatric neuro-oncology community have resulted in great strides forward in our understanding of the biology of pediatric brain tumors over the past decade. This community now stands before the challenge to use this knowledge to develop effective therapies for these most devastating and difficult-to-treat childhood cancers. We hope that the research presented in this thesis will make a significant contribution to the field of pediatric neuro-oncology research and aid the international scientific community in facing this great challenge.